JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Fractionation, Characterization, and Study of the Emulsifying Properties of Corn Fiber Gum

MADHAV P. YADAV,* NICHOLAS PARRIS, DAVID B. JOHNSTON, AND KEVIN B. HICKS

Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038

Corn fiber gum (CFG) has been fractionated by hydrophobic interaction chromatography on Amberlite XAD-1180 resin using ionic, acidic, basic, and hydrophobic solvents of different polarities. Characterization, including determination of total carbohydrate, acidic sugar, and protein content, has been done for each fraction together with measurements of molar mass, polydispersity, radius of gyration, Mark–Houwink exponent, and intrinsic viscosity using multiangle laser light scattering and online viscosity measurements. Emulsification properties of all fractions in an oil-in-water emulsion system with 20:1 oil to gum ratio were studied by measuring turbidity over 14 days. The results indicate that CFG consists of different components differing in their molecular weights and carbohydrate and protein contents. The main fraction eluted with NaCl, although low in protein content, has the highest average molecular weight and was determined to be a better emulsifier than the other fractions. The unfractionated CFG, which contains different molecular species, is the best emulsifier.

KEYWORDS: Corn fiber; arabinoxylan; corn fiber gum; emulsification; homogenization

INTRODUCTION

Corn fiber gum (CFG) is an arabinoxylan (a hemicellulose B) that can be extracted from corn fiber, a low-value byproduct of corn wet- and dry-milling processes that consists of the pericarp and endosperm fiber of the corn kernel. It is produced in large quantities during the corn wet-milling process, which fractionates corn kernels into starch, oil, fiber, and protein streams. It is used mainly as an ingredient in animal feeds and exported to Europe as corn gluten feed (corn fiber mixed and dried with corn steep liquor). Because corn gluten feed has a relatively low economical value, there has been a strong interest in finding more valuable products from corn fiber. In recent years, research into finding more valuable products from corn fiber has intensified due to the significant increase in the production of fuel ethanol from corn. The new commercial products produced from low-valued coproducts could help to significantly reduce the cost of fuel ethanol.

Hemicellulose is the major (38–57%) and most unique component of corn fiber followed by starch (10–30%), protein (15%), cellulose (15%), oil (3.7%), and other substances such as lignin and ash (3%) (*1*). In general, hemicellulose is defined as an alkali-soluble polymer from plant cell walls (*2*), and the hemicellulose B isolated from corn fiber is generally composed of D-xylose (48–54%), L-arabinose (33–35%), galactose (7–11%), and glucuronic acid (3–6%) (*3*). The hemicelluloses, in general, have been poorly utilized by industry, although they comprise about 25–35% (depending upon the source) of plant material (*4*). If CFG can be produced economically, it has a number of potential industrial applications. It could be utilized as a viscosity modifier, adhesive, thickener, flavor binder, a film former, or an emulsifier (*5, 6*).

It is well documented that gum arabic has excellent emulsifying properties for beverage emulsion systems and that its protein (*7*) and lipid (*8, 9*) components play an important role in its emulsification properties. It is widely used in the soft drink industry (*10*) to stabilize flavor oil emulsions in water. Our previous studies have clearly indicated that CFG can be a potential gum arabic replacer for oil-in-water emulsion systems (*3*). Its emulsion-stabilizing capacity also correlates well with its protein and lipid contents (*3, 11*). Our study also showed that CFG with a higher molecular weight had a better emulsion-stabilizing capacity than the lower molecular weight fractions (*12*). The aim of the present investigation is to fractionate CFG by hydrophobic affinity chromatography, characterize all fractions, and evaluate their emulsifying properties using a citrus oil emulsion in water.

MATERIALS AND METHODS

Materials. The oven-dried "fine" corn fiber (fiber originating from the endosperm portion of corn kernels) sample was kindly provided by ADM Research. It was ground to a 20-mesh particle size using a Wiley mill and deoiled by extracting with hexane (*13*). Starch was removed by heat-stable Termamyl α-amylase (Novozymes, Inc., Davis, CA) treatment (*14*). Amberlite XAD-1180, a polymeric adsorbent, was purchased from Acros Organic.

* Author to whom correspondence should be addressed [telephone (215) 836-3783; fax (215) 233-6406; e-mail madhav.yadav@ars.usda.gov.

10.1021/jf703672d
Published on Web 05/20/2008

Isolation of Corn Fiber Gum. CFG was isolated from deoiled and destarched fine corn fiber according to the alkaline hydrogen peroxide procedures of Doner et al. (*14*). In brief, deoiled and destarched corn fiber was mechanically stirred into an alkaline solution containing 1 mequiv each of NaOH and $Ca(OH)_2$ per gram of fiber in the extraction medium and boiled for 1 h. The residue obtained after centrifugation was resuspended in water, boiled for 5 min, and centrifuged again. The combined supernatant was treated with H_2O_2 at pH 11.5 for 2 h, and then the pH was adjusted to 4.0−4.5 to precipitate hemicellulose A (Hemi A). The supernatant was treated with 2 volumes of ethanol to precipitate hemicellulose B (CFG), collected, and dried in a vacuum oven.

Fractionation of Corn Fiber Gum Using Hydrophobic Interaction Chromatography (HIC). CFG was fractionated by HIC on Amberlite XAD-1180, a large-pore, high-surface area, nonionic, hydrophobic, cross-linked polystyrene polymeric resin. It develops its adsorptive properties from its macroreticular structure, high surface area, and the polyaromatic nature of its surface and adsorbs hydrophobic molecules from polar solvents. The resin was sequentially washed with water, methanol, and water and packed in a glass column (dimensions 4 × 50 cm) with 0.5 M NaCl and 0.1% TFA. The CFG sample (1.0 g) was dissolved in 150 mL of 0.5 M NaCl and 0.1% TFA and stirred at room temperature overnight for hydration and complete dissolution. The sample solution was centrifuged at 5000*g* to remove any insoluble materials. The supernatant was passed through the column by gravity at a flow rate of ∼15 mL/min. The sample was eluted with 0.5 M NaCl and 0.1% TFA, and 45 mL fractions were collected. An aliquot from each fraction was assayed for total carbohydrate by phenol−sulfuric acid assay (*15*) and protein by UV absorption at 280 nm. When the elution of carbohydrate and proteinous material was complete, the column was washed with water and further eluted with 50% MeOH. In each case, 45 mL fractions were collected and monitored for carbohydrate and protein as described above. The fractions corresponding to each peak were combined, dialyzed against deionized water, and then freeze-dried and weighed. Alternatively, 1.0 g of CFG sample was fractionated batchwise on Amberlite XAD-1180 resin on a Büchner funnel by washing sequentially with 0.5 M NaCl containing 0.1% TFA, water, and 50% methanol.

In the above two trials, the yields of fractions eluted from the column showed that more than 50% of the loaded sample was retained on the resin (discussed under Results and Discussion). The resin conditioning and fractionation procedures were modified as follows: Prior to use, the resin was sequentially washed with methanol, water, 1 M NaOH, water, 0.25 M HCl, water, methanol, and water and then resuspended in water. The same glass column was packed with the well-conditioned and washed resin using deionized water. The column was backwashed by connection from its bottom to the deionized water line and up-flowing water very slowly to remove any air pockets trapped in the resin. The column was then equilibrated with 1 L of the starting column eluting solution (0.5 M NaCl, 0.1% TFA). A 1.1 g sample of CFG was prepared in 100 mL of 0.5 M NaCl, 0.1% TFA as above and passed down the column under gravity at a flow rate of ∼15 mL/min. The column was eluted with 0.5 M NaCl and 0.1% TFA under gravity, and 45 mL fractions were collected. As in the first trial, fractions were monitored by the phenol−sulfuric acid assay for carbohydrate and UV absorption at 280 nm for protein. Once no further material was detected with 0.5 M NaCl and 0.1% TFA elution (fractions 1–25), the column was eluted with water (1 L, fractions 26–50), 0.5 M NaOH (1 L, fractions 51–74), water and 0.1 M HCl (1 L, fractions 75–97), and water and 50% MeOH (1 L, fractions 98–121). The fractions corresponding to each peak were collected, extensively dialyzed, freeze-dried, and weighed.

Determination of Protein Content. The total protein content was estimated by the bicinchoninic acid (BCA) assay (*16*) using the Micro BCA protein assay reagent kit supplied by Pierce Chemical Co. (Rockford, IL). Bovine serum albumin (BSA) was used as the standard.

Gel Electrophoresis. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) of proteins in the CFG fractions was carried out on a Phast System Pharmacia (Piscataway, NJ) with a Phast gel of 20% acrylamide. Dried samples were solubilized in 200 μL of protein–solvent system (0.44 M Tris, 1 mM EDTA, 10% SDS, pH 8.0) plus 40 μL of 2-ME, and the mixtures were heated at 100 °C for 10 min. Gels were stained with 0.2% (w/v) Coomassie R350 dye. Molecular weight standards (Invitrogen Corp., Carlsbad, CA) and their corresponding molecular weights were as follows: phosphorylase, 98 kDa; bovine serum albumin (BSA), 62 kDa; glutamic dehydrogenase, 49 kDa; alcohol dehydrogenase, 38 kDa; carbonic anhydrase, 28 kDa; myoglobin red, 17 kDa; lysozyme, 14 kDa; aprotinin, 6 kDa; and insulin, B chain, 3 kDa.

Estimation of Total Carbohydrate. The total carbohydrate was determined by the colorimetric phenol−sulfuric acid assay (*15*) using xylose as the standard. A sample solution containing about 5–40 ug of carbohydrate was taken into a clean glass test tube (16 × 125 mm), and water was added to make a total volume of 500 μL. Then 12.5 μL of 80% (w/w) phenol reagent and 1.25 mL of concentrated H_2SO_4 were added and thoroughly mixed by vortexing. The tubes were allowed to stand at room temperature for ∼30 min, and the absorbance was measured at 485 nm using a Shimadzu UV spectrophotometer against a blank containing water and all of the reagents except any sugar. The amount of carbohydrate in the sample was estimated by comparison with the calibration curve of xylose standard.

Estimation of Uronic Acid Content. The uronic acid content was determined by using the modified sulfamate/*m*-hydroxydiphenyl assay of Tullia et al. (*17*). An aliquot of the sample containing up to 200 nmol of glucuronic acid was taken in a 4 mL screw-cap glass vial, and water was added to make a total volume of 0.4 mL. Forty microliters of 4 M sulfamic acid−potassium sulfamate (pH 1.6) was added. After through mixing, 2.5 mL of 75 mM sodium tetraborate in concentrated H_2SO_4 was added, vortexed, and heated in a boiling water bath at ∼100 °C for 20 min, with the tubes capped with Teflon-lined screw caps. The tubes were quickly cooled to room temperature by placing them in an ice–water bath, and then 80 μL of 0.15% (w/v) *m*-hydroxydiphenyl in 0.5% (w/v) NaOH was added and again mixed well by vigorous vortexing. In about 5–10 min the pink color development was complete, and was stable for about an hour. The absorbance of the pink color was measured at 525 nm using a Shimadzu UV spectrophotometer. The amount of uronic acid present was determined from a calibration curve constructed using a standard glucuronic acid solution.

Determination of Sugar Composition. Sugars were analyzed by HPAEC-PAD using methanolysis (*18, 19*) combined with TFA hydrolysis as explained in detail by Yadav et al. (*20*).

High-Performance Size Exclusion Chromatography (HPSEC). A solution of 2 mg/mL of CFG was prepared for chromatography by slowly adding 20 mg of gum sample with vigorous stirring into 10 mL of 50 mM $NaNO_3$ solution (mobile phase for chromatography) at room temperature to make a homogeneous solution. The gum solution was dialyzed against 50 mM $NaNO_3$, 1 L (the dialysis solution was changed four times), using 6000–8000 MW cutoff dialysis tubing. The dialyzed solution was centrifuged at 50000*g* for 10 min at 35 °C and filtered through a 0.22 μm sterile Millex-HV filter (Millipore Corp., Bedford, MA).

The chromatographic system consisted of high-performance size exclusion columns and online molar mass and viscometer detectors. The flow rate for the solvent delivery system, model 1100 series degasser, autosampler, and pump (Hewlett-Packard Corp.), was 0.7 mL/min. The samples were run in triplicate by injecting 200 μL of sample solution and eluting the columns with 50 mM sodium nitrate. The HPSEC system was composed of two PL Aquagel OH-60 and one OH-40 columns (Polymer Laboratories, Amherst, MA) in series set in a water bath at 35 °C. The chromatograph was fitted with a Dawn DSP multiangle laser light scattering photometer (MALLS) (Wyatt Technology, Santa Barbara, CA), model H502 C differential pressure viscometer (DPV) (Viscotec Corp., Houston TX), and an Optilab DSP interferometer (RI) (Wyatt Technology). Electronic outputs from the 90 °C light scattering, DPV, and RI were sent to one directory of a computer for processing with TRISEC software (Viscotek Corp.). Electronic outputs from all of the scattering angles measured by the MALLS, DPV, and RI were sent to a second directory for processing with ASTRA software (Wyatt Technology).

Emulsion Preparation. Our CFG stock solution (2.6316 mg of CFG/g of solution) containing 0.1% (w/w) sodium benzoate (a

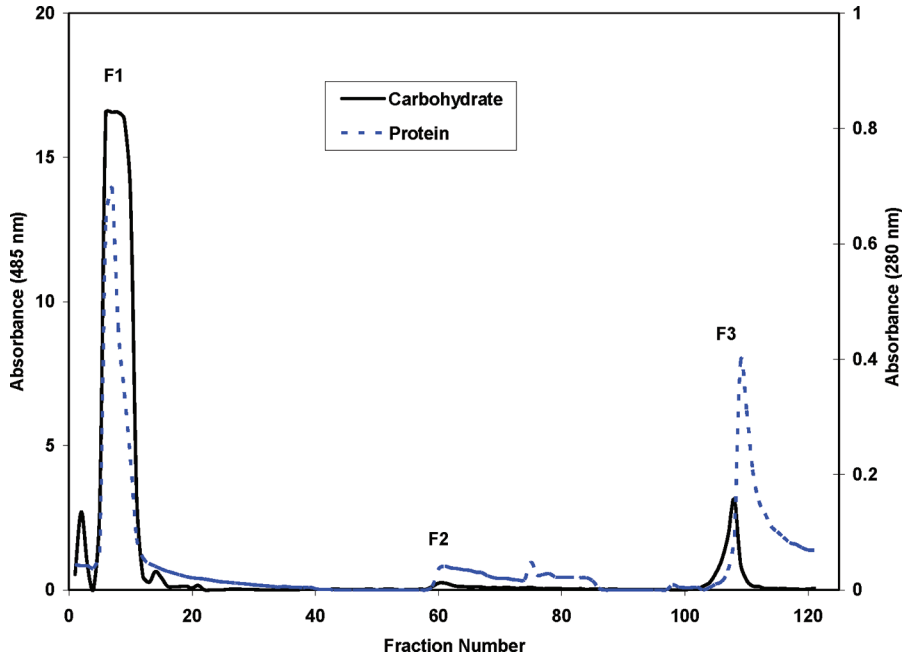

Figure 1. Elution profile of CFG fractionated by hydrophobic interaction chromatography on Amberlite XAD-1160. The stepwise eluting solvents were 0.5 M NaCl (F1, fractions 1–25), deionized water (fractions 26–50), 0.5 M NaOH (F2, fractions 51–75), deionized water washing (nothing was eluted, detected by carbohydrate and protein assay), 0.1 M HCl (fractions 76–98), and 50% methanol (F3, fractions 99–121). Solid line, neutral sugar detected with phenol/sulfuric acid assay; broken line, UV absorption at 280 nm. Fractions collected were F1−F3 (see **Table 1**).

preservative) and 0.3% (w/w) citric acid was prepared for emulsification study by slowly adding the calculated amount of CFG sample a little at a time with vigorous stirring to a solution of sodium benzoate and citric acid in water at room temperature and then gently stirring overnight to produce a hydrated, well-dissolved, and homogeneous solution.

The samples for oil-in-water emulsions were prepared in triplicate for each sample by taking 2.375 g of the above CFG stock solution (6.25 mg of CFG) and 125 mg of Valencia orange oil (total 2.5 g of solution) in a glass vial. No weighting agent was added to avoid the effects of such agent on the emulsification process. The solution was vortexed and then prehomogenized using a polytron benchtop homogenizer equipped with a 12 mm diameter head (Brinkmann, Switzerland, PT 10/35) at 15000 rpm for 30 s. The above prehomogenized emulsion was passed through the EmulsiFlex-B3 high-pressure homogenizer (Avestin Inc., Ottawa, Canada) at 10000 psi homogenization pressure three times. The resulting emulsion concentrate was diluted 31.25 times to 78.125 g in a 10.0% (w/w) sucrose solution containing 0.1% (w/w) sodium benzoate and 0.3% (w/w) citric acid. Both the polytron homogenizer probe and high-pressure homogenizer chamber were rinsed with ~2.5 mL of sugar solution, three times (saved from the total solution for this purpose). Each time the polytron homogenizer probe was dipped into the solution and run for a few seconds, and then that solution was passed through the high-pressure homogenizer as above. After these three rinses, the high-pressure homogenizer was rinsed with one passage of air to blow the remaining traces of liquid out of the sample chamber. All three rinses were combined into the 75.625 g total solution in a 125 mL glass bottle and set on the bench at room temperature.

Measurement of Emulsifying Properties by Turbidity Measurement. The emulsion stability was measured as turbidity (*21*) with modification as we explained in our previous paper (*3*): $T = 2.303AD/l$, where T = turbidity in 1/cm, A = observed absorbance at 650 nm, D = dilution factor, and l = path length of the cuvette in cm. The turbidity was determined immediately after preparing the dilute emulsion by measuring the absorbance at 650 nm using a UV-1700 spectrophotometer (Shimadzu, Columbia, MD). The emulsion stability at each concentration was determined by emulsion breakage, which was monitored by absorbance (loss of turbidity) measurement at 650 nm against 10.0% sugar solution containing 0.1% sodium benzoate and 0.3% citric acid for 14 days.

Table 1. Percent Yield of Fractions Recovered from Hydrophobic Interaction Chromatography Column

elution medium	fraction	yield (wt %)[a]
0.5 M NaCl	F1	65.4
0.5 M NaOH	F2	2.7
50% methanol	F3	11.1

[a] Weight percent of fractions from HIC.

RESULTS AND DISCUSSION

Fractionation Using a Hydrophobic Interaction Column. For the fractionation of CFG by HIC on Amberlite XAD-1180 resin, we found it necessary to thoroughly clean the resin with acid, base, and a nonpolar organic solvent to obtain satisfactory results. The yields of the fractions eluted with 0.5 M NaCl, water, and 50% methanol from the column loaded with water and methanol washed resin were only 35.7, 0, and 7.4%, respectively (43.1% of the total CFG sample loaded on the column). It clearly indicated that the resin conditioning step, which involved water, methanol, and water, resulted in poor mass recovery. The alternative batchwise washing of the sample from the similar resin on the Büchner funnel gave only 44.9% total yield of all fractions. In both cases the recovery of the sample was <45%, indicating that more than half of the sample was retained on the column. Therefore, the resin cleanup and fractionation procedure was modified. The resin was thoroughly cleaned by sequential washing with methanol, water, alkali, water, acid, and water. After backwashing with water, it was resuspended in the first eluting solution (0.5 M NaCl) and loaded on the column. The CFG sample was fractionated on HIC as explained under Materials and Methods. The fractionation pattern of CFG on the resin after cleanup is shown in **Figure 1**. The fractions were monitored by phenol−sulfuric acid assay for carbohydrate and UV absorption at wavelength 280 nm for protein content. The combined yield of all fractions eluted from the column was 79.2% of the CFG sample loaded (**Table 1**).

Table 2. Chemical Analysis of Corn Fiber Gum and Its Fractions[a]

sample	neutral sugar (wt %)	acidic sugar (wt %)	protein content (wt %)
whole CFG	65.34 ± 1.35	6.57 ± 0.83	4.09 ± 0.07
F1	74.14 ± 4.53	5.10 ± 0.11	1.15 ± 0.01
F2	38.94 ± 2.57	6.30 ± 0.08	4.87 ± 0.02
F3	11.96 ± 0.79	1.53 ± 0.15	31.07 ± 0.49

[a] Each entry is the average of three analyses ± standard deviation.

Table 3. Carbohydrate Composition of Corn Fiber Gum Fractions (Mole Percent)

sugar[a]	whole CFG	F1	F2	F3
Ara	33.41	39.33	34.33	27.11
Gal	6.27	5.52	7.62	23.63
Glc	1.23	0.90	3.90	3.57
Xyl	54.74	50.37	49.90	43.09
GlcA	4.35	3.87	4.19	2.60
total	100	100	100	100

[a] Ara, arabinose; Gal, galactose; Glc, glucose; Xyl, xylose; GlcA, glucuronic acid.

The total carbohydrate, uronic acid, and protein contents of the whole gum and the isolated fractions are given in **Table 2**. After resin cleanup, fraction F1, the material passed down the hydrophobic column without absorbing, represented 65.3% of the total weight of CFG sample loaded and was found to contain less protein and more carbohydrate than the whole CFG and the other isolated fractions. Fraction F2, which had initially adsorbed onto the column material but was desorbed by 0.5 M NaOH, accounted for only 2.7% of total weight of CFG. This fraction was higher in protein and acidic sugar contents than fraction F1, but it was lower in the total carbohydrate content than both the whole CFG and fraction F1. Fraction F3, which was desorbed from the column on elution with 50% methanol, accounted for 11.1% of total CFG. It had higher protein content (31%), but was lower in acidic sugar and total carbohydrate contents than the whole CFG and fractions F1 and F2. It seems obvious that F3 contained some hydrophobic group making it to stick to HIC and eluting only with 50% methanol. This fraction has higher protein and lower carbohydrate contents than the other fractions.

Carbohydrate Composition. The sugar composition of whole CFG and its fractions from HIC are shown in **Table 3**. Fractions F1 and F2, eluted by 0.5 M NaCl and 0.5 M NaOH, respectively, do not differ significantly in their general sugar composition from the whole CFG. Both fractions have a typical arabinoxylan structure with similar Ara/Xyl ratios. As reported in our previous publications (*3, 20*), they contain 33–39% Ara, 50–55% Xyl, 5–8% Gal, 1–4% Glc, and about 4% GlcA, but the sugar composition of F3 eluted by 50% MeOH differs significantly from the whole CFG and other two fractions F1 and F2. Its carbohydrate molar ratio differs from the whole CFG and other fractions, showing remarkable change in its overall typical corn arabinoxylan structure.

Molecular Characterization. The weight-average molecular weight (M_w), polydispersity, (M_w/M_n), and z-average root-mean-square radius of gyration (R_{gz}) of the whole CFG and its fractions F1−F3 from HIC determined by methods MALLS and light scattering and viscometry (LSV) are given in **Table 4**. The main fraction F1, which was not absorbed to the column, has slightly higher weight-average molecular weight and lower polydispersity and radius of gyration than the whole CFG by both methods. As reported previously (*12*), there are some discrepancies in molar mass and radius of gyration determined by both MALLS and LSV methods, in which the former gives slightly higher values than the latter. The lower polydispersity of fraction F1, which constitutes the bulk of CFG, is a good indication that this main fraction contains a very homogeneous population. A very small radius of gyration (33.8 and 28.8 by MALLS and LSV methods, respectively) of such a high molecular weight (300 and 272 kDa by MALLS and LSV methods) of F1 indicates a highly branched and very compact structure. Fraction F2 eluted with 0.5 M NaOH does not look too much different from the whole gum except that it has slightly lower M_w by MALLS method than the whole gum. Because it was eluted with alkaline solution, it is likely that it adsorbed to the column due to the presence of some methylated carboxylic acid groups. The M_w values of fraction F3 eluted by 50% ethanol (9.3 and 7.0 kDa by MALLS and LSV, respectively) are about 32−37 times lower than the whole gum. This lower M_w fraction constitutes about 11% of the total population of whole gum (**Table 1**). Its low polydispersity (1.17) indicates that it is a very homogeneous fraction. The radius of gyration of F3 measured by only LSV is very low (2.49 nm) and cannot give any solid conclusion about its branching and compactness (usually an R_{gz} value of <20 nm cannot be measured with this method very accurately).

Table 4. Molar Mass, Polydispersity, and Radius of Gyration of Corn Fiber Gum Fractions

	$M_w \times 10^3$		M_w/M_n	R_{gz} (nm)	
sample	MALLS[a]	LSV[b]	MALLS[a]	MALLS[a]	LSV[b]
whole CFG	296 ± 5	258 ± 1	1.74 ± 0.01	35.7 ± 0.3	29.4 ± 0.2
F1	300 ± 3	272 ± 3	1.42 ± 0.02	33.8 ± 0.1	28.8 ± 0.2
F2	278 ± 11	259 ± 5	1.68 ± 0.04	34.5 ± 0.6	27.9 ± 0.5
F3	9.3 ± 0.2	7.0 ± 0.1	1.17 ± 0.001	ND	2.49 ± 0.09

[a] Determined by multiangle light scattering method. [b] Determined by a combination of light scattering at 90° and viscometry method.

Table 5. Mark−Houwink Exponent and Intrinsic Viscosity of Corn Fiber Gum Fractions

	a		η_w	
sample	MALLS[a]	LSV[b]	MALLS[a]	LSV[b]
whole CFG	0.64 ± 0.03	0.68 ± 0.02	1.71 ± 0.03	1.84 ± 0.01
F1	0.58 ± 0.01	0.64 ± 0.01	1.88 ± 0.01	1.98 ± 0.02
F2	0.61 ± 0.05	0.65 ± 0.05	1.65 ± 0.1	1.80 ± 0.04
F3	1.30 ± 0.09	0.99 ± 0.01	0.09 ± 0.01	0.06 ± 0.01

[a] Determined by multiangle light scattering method. [b] Determined by a combination of light scattering at 90° and viscometry method.

Fraction F1 showed a slightly lower Mark–Houwink exponent a by both MALLS and LSV methods than the corresponding whole CFG (**Table 5**), indicating a more compact structure. This agrees with our previous paper (*12*) that the CFG fractions with higher M_w showed lower a, indicating higher compactness. It can be interpreted that the highly branched structure of corn kernel arabinoxylans (*22–25*) aggregate well and occupy less space per molar mass unit, giving very compact structure. Although fraction F3 has the lowest M_w, it has the highest a, showing less branched structure than the other fractions. The weight-average intrinsic viscosities (η_w) of whole CFG and its fractions determined by both MALLS and LCV methods (**Table 5**) are well correlated to their M_w. These results agree very well with the previous studies (*12, 26, 23*), which showed that the higher M_w fraction was slightly more viscous than the lower M_w one.

Emulsifying Properties. The whole CFG and its fractions from HIC were assayed for the change in emulsion stabilities in an oil-in-water emulsion system by monitoring the turbidity

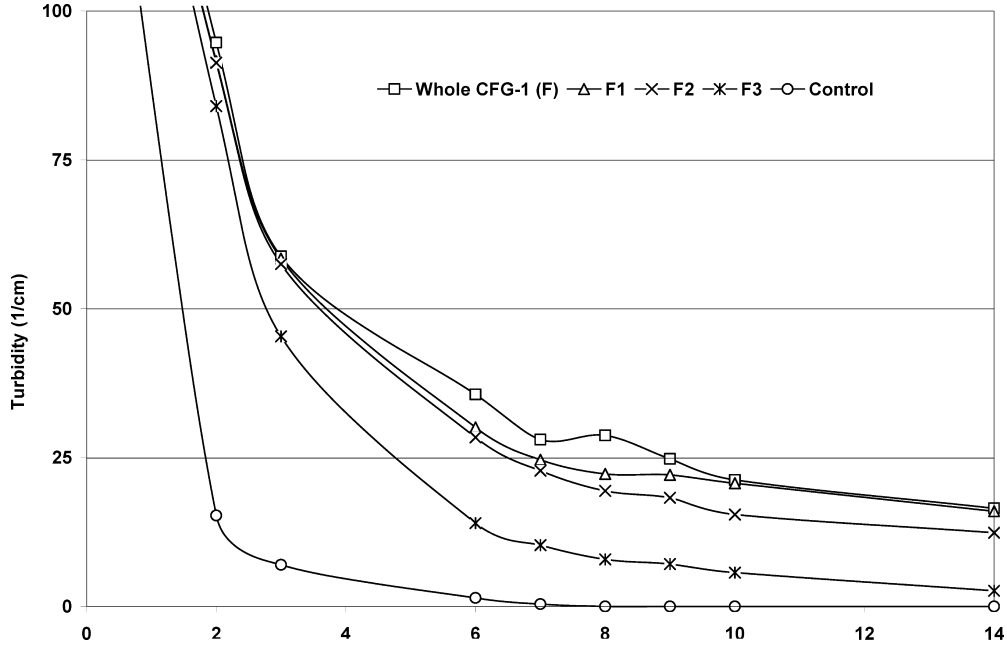

Figure 2. Emulsion stability (turbidity) of whole corn fiber gum and its fractions obtained from separation on hydrophobic interaction column at room temperature with 1:20 gum to oil ratio. The control was the emulsion prepared without any emulsifier. Turbidity was measured by taking an aliquot from the bottom of the diluted solution for 14 days. Higher turbidity indicates a greater emulsifying capacity. Each plotted point is the average of three trials ± standard deviation.

of diluted emulsions over a period of time by measuring absorbance at 650 nm and converting it to turbidity as explained under Materials and Methods. The higher turbidity is an indication of a better emulsion stability. This method is simple, rapid, and correlates well with emulsion particles sizing methods and is recommended for fast and routine analysis (*27*). **Figure 2** shows the change in turbidities of emulsions containing 6.25 mg of CFG or CFG fractions and 125 mg of oil (gum to oil ratio 1:20) for 14 days. The emulsion without any emulsifier was used as a control. The turbidity of all samples decreased for a week, and after that, they become almost constant, but the turbidity of the control solution without emulsifier reached zero in a week. The turbidities graph (**Figure 2**) shows that the whole CFG and all its fractions have some emulsion-stabilizing capacity, but the emulsion-stabilizing capacity of whole CFG is better than that of its fractions, showing that all components of CFG have some activity and that they all make some contribution to its emulsifying properties. Although the main fraction F1 (eluted with 0.5 M NaCl) was lower in protein content, its molecular weight was higher than that of other fractions. The emulsion stability of this main fraction is superior to that of all other fractions and looks very close to the unfractionated CFG. Fraction 3 is a poor emulsifier, and it has the lowest molecular weight materials (**Table 4**) consisting of low carbohydrate content and differing in its sugar composition from a typical corn arabinoxylan and other fractions. Even though this fraction has a protein content higher than other fractions (**Table 2**), it yielded an extremely poor emulsion in comparison to other fractions (**Figure 2**). This clearly demonstrates and supports our previous statement (*3, 12*) that in addition to protein content the typical molecular structure and high molecular weight of CFG are responsible for its good emulsifying properties. A similar correlation between M_w and emulsion stability of gum arabic was reported by Aoki et al. (*28*) and Dickinson et al. (*29*). It seems likely that the high molecular weight polymers adsorb on the oil droplets and form thick layers, which give an effective long-lasting steric stability to emulsion by preventing contact between tiny oil droplets. This mechanism retards coalescence and the rate of de-emulsification. These results also agree with Dickinson et al. (*30*), who found that a mixture of high and low molecular weight gums may be best for good functionality. Thus, it looks like nature has made CFG an excellent emulsifier with a good balance of molecular species, differing in size and composition, which on fractionation loses some of its functionality.

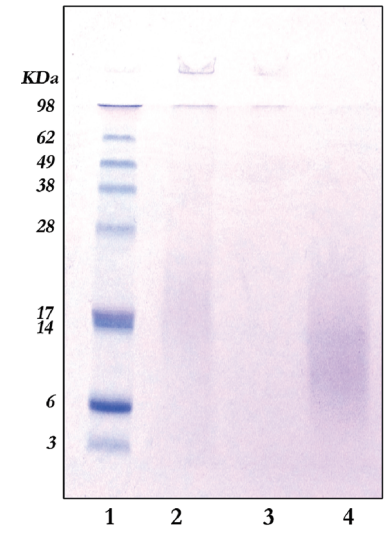

Figure 3. Gel electrophoresis SDS-PAGE of corn fiber gum (CFG) and its fractions eluted from HIC: lane 1, protein molecular weight standards; lane 2, proteins in whole CFG; lane 3, proteins eluted with 0.5 M NaCl (F1); lane 4, proteins eluted with 50% MeOH (F3).

Gel Electrophoresis. Figure 3 shows the gel electrophoresis of the whole CFG sample and its fractions from the HIC. The results indicated the presence of a group of protein bands with molecular weights centered around 20 kDa and higher molecular weight protein ~98 kDa or aggregated protein (**Figure 3**, lane 2) that did not enter the separating gel. Fraction F1, eluted with

0.5 M NaCl from the HIC column, did not show any protein band around 20 kDa but showed higher molecular weight protein in the stacking gel and at the front of the separating gel (**Figure 3**, lane 3). A significant amount of protein was seen in fraction F3, which was eluted with 50% (v/v) methanol from HIC (**Figure 3**, lane 4). Fraction F3 was strongly adsorbed on the HIC column, and alcohol was required for elution, which is characteristic of prolamines such as zein, which is the principal protein found in corn. These fractionation and gel electrophoresis results clearly indicated that mixtures of hydrophobic proteins or polypeptides were associated with low molecular weight carbohydrate present in CFG. The major fraction F1 eluted with 0.5 M NaCl does not contain any low molecular weight protein, but it does have protein associated with it.

This is the first detailed report of the emulsifying behavior of various fractions of CFG obtained by HIC. All fractions contain protein in various amounts. The whole CFG, which is a combination of many molecular species of carbohydrate and protein differing in size and composition, and the main fraction F1 made a better emulsion than fraction 2 or 3 alone. These results indicate that all components of CFG contribute to its strong emulsion-stabilizing capacity. Usually, emulsion stability increases with increasing molecular weight and protein content. Therefore, it is likely that the good emulsifying properties of the main fraction F1 are due to the combination of its high molecular weight and protein content. It is interesting that although the protein content of the last fraction, F3 (lowest molecular weight), is high, it produced the least stable emulsion relative to the other fractions. Thus, it appears that in addition to protein content a typical corn arabinoxylan structure with high molecular weight may be essential to be a good emulsifier. Future work will be directed toward the emulsifying properties of different classes of protein and their covalent interaction with carbohydrate polymers.

ACKNOWLEDGMENT

We thank Hoa K. Chau and John Minutolo for their technical support, Paul Pierlott for taking the gel photograph, and Kyle Beery from ADM Research for providing the sample of corn "fine fiber".

LITERATURE CITED

(1) Osborn, D.; Chen, L. F. Corn hull hydrolysis using glucoamylase and sulfuric acid. *Starch/Staerke* **1984**, *36*, 393–395.

(2) Darvill, A.; McNeil, M.; Albersheim, P.; Delmer, D. P. In *The Biochemistry of Plants*; Stumpf, P. K., Conn, E. E., Eds.; Academic Press: New York, 1980; pp 91–140.

(3) Yadav, M. P.; Johnston, D. B.; Hotchkiss, A. T.; Hicks, K. B. Corn fiber gum: a potential gum arabic replacer for beverage flavor emulsion. *Food Hydrocolloids* **2007**, *21*, 1022–1030.

(4) Prade, R. A. Xylanases: from biology to biotechnology. *Biotechnol. Genet. Eng. Rev.* **1996**, *13*, 101–131.

(5) Whistler, R. L. Hemicelluloses. In *Industrial Gums, Polysaccharides and Their Derivatives*, 3rd ed.; Whistler, R. L., BeMiller, J. N., Eds.; Academic Press: San Diego, CA, 1993; pp 295–308.

(6) Woo, D.-H. Stabilization of the emulsion prepared with dietary fiber from corn hull. *Food Sci. Technol.* **2001**, *10* (4), 348–353.

(7) Randall, R. C.; Phillips, G. O.; Williams, P. A. The role of the proteinaceous component on the emulsifying properties of gum arabic. *Food Hydrocolloids* **1988**, *2*, 131–140.

(8) Yadav, M. P.; Johnston, D. B.; Hicks, K. B.; Nothnagel, E. A. The role of lipids and protein components in the emulsification properties of gum arabic and corn fiber gum. *Foods Food Ingred. J.* **2006**, *211* (3), 245–252.

(9) Yadav, M. P.; Nothnagel, E. A. Chemical investigation of the structural bases of the emulsifying activity of gum arabic. *Food Hydrocolloids* **2007**, *21*, 297–308.

(10) Verbeken, D.; Dierckx, S.; Dewettinck, K. Exudate gums: Occurrence, production, and applications. *Appl. Microbiol. Biotechnol.* **2003**, *63*, 10–21.

(11) Yadav, M. P.; Moreau, R. A.; Hicks, K. B. Phenolic acids, lipids, and proteins associated with purified corn fiber arabinoxylans. *J. Agric. Food Chem.* **2007**, *55*, 943–947.

(12) Yadav, M. P.; Fishman, M. L.; Chau, H. K.; Johnston, D. B.; Hicks, K. B. Molecular characteristics of corn fiber gum and their influence on its emulsifying properties. *Cereal Chem.* **2007**, *84* (2), 175–180.

(13) Moreau, R. A.; Powell, M. J.; Hicks, K. B. Extraction and quantitative analysis of oil from commercial corn fiber. *J. Agric. Food Chem.* **1996**, *44*, 2149–2154.

(14) Doner, L. W.; Chau, H. K.; Fishman, M. L.; Hicks, K. B. An improved process for isolation of corn fiber gum. *Cereal Chem.* **1998**, *75* (4), 408–411.

(15) Ashwell, G. New colorimetric methods for sugar analysis. *Methods Enzymol.* **1966**, *8*, 85–95.

(16) Smith, P. K.; Krohn, R. I.; Hermanson, G. T.; Mallia, A. K.; Gartner, F. H.; Provenzano, M. D.; Fujimoto, E. K.; Goeke, N. M.; Olson, B. J.; Klenk, D. C. Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **1985**, *150*, 76–85.

(17) Tullia, M. C.; Filisetti-Comi, C.; Carpita, N. C. Measurement of. uronic acids without interference from neutra1 sugars. *Ana1. Biochem.* **1991**, *197*, 157–162.

(18) Chambers, R. E.; Clamp, J. R. An assessment of methanolysis and other factors used in the analysis of carbohydrate-containing materials. *Biochem. J.* **1971**, *125*, 1009–1018.

(19) Svetek, J.; Yadav, M. P.; Nothnagel, E. A. Presence of a glycosylphosphatidylinositol lipid anchor on rose arabinogalactan proteins. *J. Biol. Chem.* **1999**, *274*, 14724–14733.

(20) Yadav, M. P.; Johnston, D. B.; Hicks, K. B. Structural characterization of corn fiber gum from coarse and fine corn fiber and a study of their emulsifying properties. *J. Agric. Food Chem.* **2007**, *55*, 6366–6371.

(21) Pearce, K. N.; Kinsella, J. E. Emulsifying properties of proteins: evaluation of a turbidometric technique. *J. Agric. Food Chem.* **1978**, *26*, 716–723.

(22) Montgomery, R.; Smith, F. Structure of corn hull hemicellulose. III. Identification of the methylated aldobiuronic acid obtained from methyl corn hull hemicellulose. *J. Am. Chem. Soc.* **1957**, *79*, 695–697.

(23) Saulnier, L.; Marot, C.; Chanliaud, E.; Thibault, J.-F. Cell wall polysaccharide interactions in maize bran. *Carbohydr. Polym.* **1995**, *26*, 279–287.

(24) Whistler, R. L.; Corbett, W. M. Oligosaccharides from partial hydrolysis of corn fiber hemicellulose. *J. Am. Chem. Soc.* **1955**, *77*, 628–630.

(25) Whistler, R. L.; BeMiller, J. N. Hydrolysis components from methylated corn fiber gum. *J. Am. Chem. Soc.* **1956**, *78*, 1163–1165.

(26) Fishman, M. L.; Doner, L. W.; Chau, H. K.; Hoagland, P. D. Characterization of hemicellulose B from corn fiber by high-performance size exclusion chromatography with on-line molar mass and viscometric detection. *Int. J. Polym. Anal. Charact.* **2000**, *5*, 359–379.

(27) Einhorn-Stoll, U.; Weiss, M.; Kunzek, H. Influence of the emulsion components and preparation method on the laboratory-scale preparation of o/w emulsions containing different types of dispersed phases and/or emulsifiers. *Nahrung/Food* **2002**, *46* (4), 294–301.

(28) Aoki, H.; Katayama, T.; Ogasawara, T.; Sasaki, Y.; Al-Assaf, S.; Phillips, G. O. Characterization and properties of *Acacia senegal* (L.) Willd. Var. Senegal with enhanced properties (Acacia (sen) SUPER GUM^{TM}): Part 5. The factors affecting emulsification

effectiveness of Acacia Senegal and Acacia (sen) SUPER GUM™. *Food Hydrocolloids* **2007**, *21*, 353–358.

(29) Dickinson, E.; Galazka, V. B.; Anderson, D. M. W. Effect of the molecular weight on the emulsifying behavior of gum arabic. In *Food Polymers, Gels and Colloids*; Dickinson, E., Ed.; Royal Society of Chemistry: Cambridge, U.K., 1991; pp 490–493.

(30) Dickinson, E.; Galazka, V. B.; Anderson, D. M. W. Emulsifying behavior of gum arabic. 2. Effect of the gum molecular weight on the emulsion droplet-size distribution. *Carbohydr. Polym.* **1991**, *14*, 385–392.

Received for review December 17, 2007. Revised manuscript received March 7, 2008. Accepted March 9, 2008. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

JF703672D